Gut

Leading article

Clinical relevance of hepatitis C virus genotypes

Genotypes of HCV

Hepatitis C virus (HCV) has been identified as the main causative agent of post-transfusion non-A, non-B hepatitis.[1] [2] Infection with HCV is normally persistent (>20–30 years) and is associated with the development of chronic active hepatitis and cirrhosis. Hepatic failure from advanced liver disease is a frequent indication for liver transplantation. In most countries, HCV has become one of the principal identifiable causes of hepatocellular carcinoma (HCC).

It is possible to classify HCV into a series of distinct genotypes that differ substantially in nucleotide sequence from one another and show varied geographical and epidemiological distributions. Comparisons of complete genome sequences, or of subgenomic regions such as E1, NS4 or NS5, have allowed variants to be classified into at least six major genotypes, each of which comprises a number of more closely related subtypes.[3–6] Variability is distributed throughout the genome, with the non-structural genes of different genotypes showing only 65–70% nucleotide sequence similarity. Genotypes 1, 2 and 3 are widely distributed throughout Western countries and the Far East (Japan, China, Taiwan, Thailand), whereas others show more restricted geographical distributions. For example, types 5 and 6 are largely confined to South Africa and South East Asia, respectively, whereas type 4 is found predominantly in the Middle East and in Central Africa. The underlying reasons for the geographical differences in genotype distributions are poorly understood; HCV is only efficiently transmitted by parenteral routes, yet blood transfusion and needle-sharing amongst drug misusers are historically relatively recent innovations. Although recent analyses indicate that the current genotypes of HCV evolved from a common ancestor at least 500–2000 years ago,[7] we have little insight into the mechanism of HCV transmission over this period. This century, successive waves of HCV infection have occurred in many parts of the world, including Europe, USA and the Far East, in association with new risk groups for infection. This recent spread of HCV was initially associated with types 2 and 1b, followed more recently by types 1a and 3a, the latter being most frequently found in intravenous drug misusers.[7–12] As a result, the prevalence of HCV infection has greatly increased over the past 50 years in most countries, but only now are we beginning to realise the medical consequences of this extremely slowly progressive disease.[13]

Serological diagnosis of HCV infection

Laboratory diagnosis of HCV infection is most frequently based upon the detection of antibody to HCV by ELISA. Screening of blood donations has led to a great reduction in the incidence of post-transfusion hepatitis caused by HCV. However, serological tests are necessarily indirect, and produce false negative results in acutely infected individuals before seroconversion to antibody. Transmission of HCV through blood donations collected during this relatively long "window" period remains a problem, particularly for donations collected from high risk populations and from new donors. Although serological assays for HCV have improved since their introduction in 1991, problems remain with their sensitivity and the lack of adequate confirmatory tests for reactive specimens. For example, current "confirmation" assays generally use the same antigens as those present in the screening assay.

One question that has not been adequately addressed is whether it is appropriate to screen individuals using ELISAs based upon recombinant proteins derived from only one genotype of HCV (almost invariably type 1a). Significant antigenic variation between genotypes might be expected to render current assays suboptimal for screening patient and blood donor populations who may be infected with variants other than type 1. At present, the extent to which this leads to samples being missed from this cause is unknown. Among the components of current second and third generation screening assays, antigenic variation is greatest in the non-structural proteins, such as c33c (NS3), c100 (NS4) and NS5 (NS5a).[8] [14] In contrast, the core protein amino acid sequence is highly conserved, and it is likely that this component is the most effective at detecting cross-reactivity with antibody produced to other genotypes.[8] To estimate the genotype dependence of the sensitivity of the Ortho third generation assay, we compared antibody reactivity of samples collected from blood donors infected with genotypes 1, 2 and 3. Although antibody titres varied greatly between individuals, we found an approximate fivefold reduction in median reactivity of type 2 and 3 samples in the ELISA,[15] a difference in sensitivity that can be shown experimentally to reduce substantially the number of samples that can be detected on screening (Neville *et al*, unpublished data). As a result of these findings, we are currently developing ELISAs based upon recombinant proteins from other genotypes, and comparing their effectiveness with conventional assays for the screening of blood donors from populations infected with variants other than type 1.

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Vaccine development

The degree of sequence variation between genotypes of HCV is similar to that observed between variants of other viruses, such as the four serotypes of the flavivirus, dengue virus, or between poliovirus types 1, 2 and 3. Through the use of in vitro neutralisation assays, it has been established that an antibody response to one serotype does not protect or neutralise the infectivity of another. Consequently, fully protective vaccines for dengue and polio are multivalent and contain antigens or attenuated virus strains of each of the serotypes. Although there is no comparable neutralisation assay for HCV, it would be reasonable to assume that antigenic variation, particularly in the envelope proteins, E1 and E2, will have a significant effect upon cross-neutralisation, and it is likely that an effective vaccine for HCV would also have to contain immunogens from each of the major genotypes.

Currently, a vaccine for HCV remains a distant prospect; however, a weak and transient serological response to recombinant envelope proteins can be elicited by immunisation of chimpanzees, which has been shown to protect or modulate infection from challenge with low doses of the homologous virus strain.[16] In the future, a number of issues need to be tackled, of which the most important is whether a vaccine should be designed to produce a predominantly neutralising antibody response (the current strategy) or whether antigens should be chosen to provoke cytotoxic T cell (CTL) activity. CTL responses have been identified as the principal mechanism for protective immunity from infection with simian immunodeficiency virus, a model used for the development of a vaccine to HIV-1. A comparable vaccine for HCV containing proteins or peptides corresponding to highly conserved T cell epitopes in the core and NS3 regions might induce a more broadly cross-protective response than a vaccine based upon envelope proteins.

Disease progression

For most RNA viruses, the existence of extensive sequence differences between serotypes has remarkably little effect on the phenotype of a virus, other than in its antigenic properties (see earlier). For example, poliovirus types 1, 2 and 3 seem to be equally infectious and equally likely to cause paralytic disease by spread into the nervous system. Similarly, different serotypes of dengue virus show similar propensities to cause viral haemorrhagic fever. On the basis of these and observations of other RNA viruses, there would be no logical reason to suspect the existence of major differences between genotypes of HCV in their clinical course or disease associations.

This issue has been extensively investigated, mainly taking the form of cross-sectional studies where the frequencies of infection with different genotypes are compared among patients with different disease outcomes, such as the development of cirrhosis, HCC and autoimmune disease – for example, mixed essential cryoglobulinaemia. These studies have frequently produced conflicting results; five major studies published since 1994 concluded that type 1b was no more likely to cause cirrhosis than other genotypes,[11] [12] [17–19] whereas six similarly conducted investigations found a significantly greater proportion of type 1b infection among cirrhotic patients.[8] [20–24] These and other comparisons have found increased alanine aminotransferase (ALT) levels in patients infected with type 1b compared with type 2 HCV, although this was not necessarily associated with more severe disease on examination of biopsy specimens.[25–27] However, these investigations may have been affected by different epidemiological characteristics of infection with

different genotypes that cannot be adequately corrected for using multivariate analysis. For example, it could be argued that the higher frequency of cirrhosis in type 1 infected individuals results from a longer mean duration of infection than other genotypes. For this reason, disease associations of type 3 are particularly difficult to compare because of its association with intravenous drug misuse and infection in a younger age group.[8–12] However, people infected with type 1b and type 2 HCV in Japan have been consistently shown to have similar age distributions and risk factors yet often differ in stage of liver disease.[28–30]

There is a greater consensus that infection with type 1b predisposes towards the development of HCC,[23] [31–34] with only two negative or contrary reports where significant numbers have been analysed.[30] [35] There are also several reports that liver transplantation of type 1b infected patients is associated with a higher rate of active disease after transplantation and graft destruction.[36–38] On the basis of current knowledge, it is still difficult to conclude whether type 1b (or type 1 generally) has a greater pathogenic potential than other genotypes, Unfortunately, controlled prospective investigation of disease progression among patients with known durations of infection is hampered by the extremely slow course of disease.

Treatment

Alpha or lymphoblastoid interferon has been investigated extensively as a treatment for HCV infection. In the past, response to treatment was generally assessed by sustained normalisation of ALT levels and improvement in liver histology after treatment. More recently, PCR has been used to monitor the disappearance of viraemia. Generally, these measures of outcome are concordant. Based upon the results of 40 published studies, representing the collective experience of treating at least 3540 patients, significant differences in response rates between genotypes have been observed in 37. In a typical study,[38a] sustained normalisation of ALT/clearance of viraemia was achieved in only 11% of type 1 infected patients compared with response rates of 60 and 33% for types 2 and 3. Higher rates of response, particularly among those infected with type 1, have been achieved by high dose interferon administration (for example, 6 megaunits three times a week for 12 months),[39] [40] or combination with ribavirin.[41] [42] Other variables that independently increase response rates are the absence of cirrhosis and low levels of circulating virus RNA.

Little is currently understood about the underlying mechanism for the observed differences in response rate. This difficulty is compounded by our current ignorance of the mechanism of action of interferon against HCV. Some experimental observations suggest a direct antiviral action, whereas others suggest that interferon is principally immunomodulatory and stimulates the immune system to clear the virus, as is the case for hepatitis B virus. It has been suggested that type 1b has a greater replicative capacity than other genotypes, possibly as a consequence of genetic differences in a region of NS5a that acts as a co-factor for RNA replication.[43] [44] Consistent with this hypothesis, there are numerous reports (at least 14) that type 1b infection is associated with higher circulating virus loads than type 2 (or type 3). However, studies using assays that are equally sensitive for virus RNA of different genotypes (for example, version 2 of the Chiron branched DNA assay), or by correction of values from bDNA version 1.0,[45] it has been shown consistently that virus loads among genotypes 1 to 6 are similar.[11] [46] [46a] These more recent findings have the necessary implication that virus load and genotype are independent predictors for response to interferon.

Pre-treatment assessment of these variables will undoubtedly permit more appropriate patient selection for treatment, or be used to calculate the necessary dose and duration to obtain a sustained response. For example, a recent Norwegian study, that assessed virus load, liver biopsy appearance and genotype before treatment, identified a group of patients with a probability of response of 83% (type 2, virus load 10^5 copies RNA/ml) to standard interferon treatment and another group with an 8% probability of response (type 1, virus load 3×10^7 copies RNA/ml).[12] In the future, pre-treatment variables such as virus genotype should be incorporated into cost-benefit analyses of HCV treatment to assist in the development of policies for more effective management of HCV infection.[47]

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